ON SOME PARASITIC PROTOZOA FROM CEYLON.

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(With Plate II.)

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INTRODUCTION.

THE following pages are the outcome of a recent visit which I made to Ceylon whilst holding the Balfour Studentship of Cambridge University. During my stay in the Island, which dated from the beginning of July until the end of September, I examined a number of animals from various parts of the country, with a view to discovering parasitic Protozoa. A good deal of my work was attended with entirely negative results, though a certain number of new forms were found, which are here recorded. Both negative and positive results, however, are given in the following pages, in order to assist future workers who may take up the investigation of Ceylon Protozoa.

The work was carried out chiefly in the laboratory attached to the Museum at Colombo, in the laboratory at the Botanic Gardens, Peradeniya, and in the resthouse at Trincomalee.

I wish here to offer my warmest thanks to those who have helped me, in one way or another, in my work—especially to Mr. E. E. Green, Government Entomologist, and to Mr. R. H. Lock, Acting Director of the Botanic Gardens, for their assistance during my stay in Peradeniya; and to Dr. Willey, the late Director of the Colombo Museum, for his unfailing aid and kindness throughout my visit. Though the results of my work here recorded are inconsiderable, they would have been far less but for Dr. Willey's assistance. His extensive knowledge of the fauna of Ceylon, and his untiring zeal in obtaining material for me, proved of incalculable value. Whatever merit attaches to the results here set forth is due in a large

measure to Dr. Willey's enthusiastic collaboration. I am glad to have this opportunity of thanking him once more, and of acknowledging my great indebtedness to him.

This paper was completed, after working through a part of the material which I brought back to England, at the Imperial College of Science and Technology, London, S.W.

I have divided the account of my work into two main parts :—

(1) A record, with notes, of the animals examined ; and
(2) A description of some new forms which I found in the course of examining these animals.

I.—RECORD OF ANIMALS EXAMINED, WITH RESULTS AND COMMENTS.

A.—Record of Animals whose Blood was examined for Protozoa.

FISHES.

With the exception of *Saccobranchus fossilis*, all the seven species of fresh-water fish, whose blood I was able to examine, proved negative.

A.—*Infected.*

1. *Saccobranchus fossilis.*—The record of the examination of the blood of this species is as follows :—

One individual from Colombo lake (part not recorded) (July). Blood negative. Twelve specimens from Colombo lake (Hunupitiya and Kollupitiya) (Sept.). Blood of all negative.

Four specimens from Nugegoda (Sept.). Blood negative.

Two specimens from Fort side of Colombo lake (Sept.). Both infected with trypanosomes. (See comments below.)

B.—*Not infected.*

2. *Anabas scandens.* One individual, Colombo (July).
3. *Clarias magur.*—One individual, Colombo (Sept.).
4. *Etroplus suratensis.*—Two individuals, Colombo lake (Sept.).
5. *Gobius giuris.*—Four specimens, Colombo lake (Sept.).
6. *Ophiocephalus punctatus.*—Two specimens, Colombo (Sept.).
7. *Ophiocephalus striatus.*—A single individual, Colombo (Sept.).

Comments.—The trypanosome found in *Saccobranchus* is that already described by Castellani and Willey (1904) under the name *Trypanosoma saccobranchi.* For the benefit of future workers who may seek this trypanosome, I would call attention to the curious distribution which it seems to have in the fishes of the Colombo lake. My experience indicates that only those fish taken from the *Fort side* of the lake are infected.

Castellani and Willey also failed to find trypanosomes in the blood of *Ophiocephalus striatus*, though they note that Lingard found trypanosomes in this species in India.

The same observers also record a trypanosome as occurring in the blood of *Gobius giuris*, although—as recorded above—the four individuals of this species which I examined proved negative.

Castellani and Willey also record a trypanosome from *Macrones cavasius*, a Silurid.

Amphibians.

The common frog, *Rana tigrina*, is the only amphibian in which I have found blood parasites.

A.—*Infected.*

1. *Rana tigrina.*—My records are as follows:—Two individuals (Colombo, July), both infected with trypanosomes and hæmogregarines. One individual (Colombo, July) infected with hæmogregarines only. Two individuals (Colombo, Aug.), blood negative. One very young specimen (Peradeniya, Aug.), blood negative. Two individuals (Colombo, Sept.), blood of both negative.

B.—*Not infected.*

2. *Bufo melanostictus.* — Three individuals (Colombo, July). One young individual (Peradeniya, Aug.).

3. *Ixalus leucorrhinus.*—A single specimen from Peradeniya (Aug.).

4. *Rhacophorus maculatus.*—One individual from Peradeniya (Aug.) and one from Trincomalee (Sept.).

Comments.—Castellani and Willey examined *R. tigrina*, with negative results. The parasites which I encountered are therefore recorded for the first time from Ceylon frogs. I have little doubt that the hæmogregarine which I found in *R. tigrina* is the same as that described from this species in Bombay by Berestneff (1903), and named *Hæmogregarina berestneffi* by Castellani and Willey (1905).* I encountered intracorpuscular individuals of various forms and sizes, many of them showing the characteristic pink-staining sheath described by Berestneff. In addition to these forms, there were also many free gregariniform individuals in the blood plasma. These were actively motile. I several times observed small forms enter red blood corpuscles. They did this by boring directly into the corpuscle, very much in the way described by Schaudinn (1903) in the case of the sporozoites of *Plasmodium vivax*, but the time taken was very different, as entry was effected in a few minutes. Occasionally, the animal, after reaching the inside of the corpuscle, rested for a few minutes and then wriggled

* Patton (1908) states that he has "had the opportunity of studying no less than five hæmogregarines in *Rana tigrina* and *Rana hexadactyla*, not only in the frogs, but in the leech which transmits them."

its way out again into the blood plasma. The curious method of entry by being engulfed by the corpuscle—recently described in detail by Neresheimer (1909) in *Lankesterella*—I never saw.

Figures of some of the forms of the hæmogregarine encountered are given in Plate II., figs. 3–8.

Berestneff also recorded a trypanosome from the Indian frogs. No name was given to it, and as I believe other observers have also seen this same parasite, which is probably the same as the one I found in Ceylon, I have contented myself with a brief description and figure of the organism, without bestowing a new name upon it. (See p. 74.)

Reptiles.

I had opportunities of examining the blood of a number of different reptiles, including crocodiles, tortoises, lizards, and snakes. The results obtained are as follows :—

Crocodiles.

I was able to examine the blood of two crocodiles, *Crocodilus porosus*. The first, a small specimen from Dadugan-oya, Veyangoda (July), contained a hæmogregarine. (See p. 79.) The second, a very young individual from Ja-ela, near Colombo (Sept.), was negative.

No hæmogregarines have been described from Ceylon crocodiles before, though several other crocodiles from other parts of the world have been found to harbour these parasites. (See p. 79.)

Tortoises.

The three following species of tortoise were examined :—

1. *Emyda vittata.*—Of five specimens examined in Colombo (July), the presence of trypanosomes could be demonstrated in only one individual, and in very small numbers.

2. *Nicoria trijuga.*—Three individuals from Colombo lake (July) : one heavily infected with hæmogregarines, one slightly infected, and one in which no parasites could be detected.

3. *Testudo elegans.*—Two individuals from Sigiriya (Sept.) showed no blood parasites.

Comments.—The trypanosome found in *Emyda vittata* is that already described by Miss Robertson under the name *T. vittatæ* [Robertson (1908) and (1909)]. The hæmogregarine from *Nicoria* is *H. nicoriæ* (Castellani and Willey, 1904).

Lizards.

Examination of the blood of twelve species of Lacertilia gave the following results :—

A.—*Infected.*

1. *Hemidactylus leschenaultii.*—At Trincomalee (Sept.) nearly every individual examined was infected with *Hæmocystidium*

simondi, Castellani and Willey. Some individuals harboured trypanosomes and hæmogregarines in addition. A single specimen from Habarana (Aug.) was infected with trypanosomes. (See remarks below.)

B.—*Not infected.*

2. *Calotes ophiomachus.*—Eight individuals (Colombo, July).

3. *Calotes versicolor.*—Twenty-five specimens from Colombo (July) and one from Peradeniya (Aug.).

4. *Ceratophora stoddartii.*—Three individuals (Peradeniya, Sept.).

5. *Hemidactylus depressus.* — Six individuals (Trincomalee, Sept.).

6. *Hemidactylus frenatus.* — A single specimen (Trincomalee, Sept.).

7. *Hemidactylus triedrus.*—One individual from Colombo (Aug.), one from Peradeniya (Aug.), and two from Trincomalee (Sept.).

8. *Lygosoma punctatum.*—Five specimens (Peradeniya, Aug.).

9. *Lyriocephalus scutatus.*—Three specimens (Peradeniya, Aug.).

10. *Mabuia carinata.*—Three individuals from Colombo (July), two from Peradeniya (Aug.), one from Colombo (Sept.), and one from Peradeniya (Sept.).

11. *Sitana ponticeriana.*—One individual (Trincomalee, Sept.).

12. *Varanus bengalensis.*—A single specimen (Trincomalee, Sept.).

Comments.—It is curious to find that all the lizards—geckoes excepted—harbour no blood Protozoa. In Europe and in Africa (*cf.*, for example, Wenyon's recent work, 1908*a*) the lizards are frequently infected with hæmogregarines, but Asiatic lizards appear to be much less frequently so. The absence of Protozoa in the blood of Indian lizards was remarked by Berestneff (1903). Since then Minchin (1907) has described a hæmogregarine (*H. thomsoni*) from a Himalayan lizard (*Agama tuberculata*), but facts with regard to the infection of other Asiatic lizards are extremely scanty.

Hæmocystidium simondi, which I found in the Trincomalee specimens of *Hemidactylus leschenaultii*, was discovered and described by Castellani and Willey (1904), and has since been observed by Miss Robertson (1908). I was fortunate enough to be able to work out a part of the life-cycle of this organism. the description of which I shall publish elsewhere.

The trypanosomes which I found were those described by Miss Robertson (1908) as *T. leschenaultii*. Another form which she observed in *H. leschenaultii* and *H. triedrus*, and named by her *T. pertenue*, I never encountered.

It is perhaps worthy of comment that I have—in common with previous workers—never found Protozoa in the blood of *Hemidactylus depressus*, although it lives in the jungle in company with the infected geckoes.

Filariæ were found in the blood of several *Calotes versicolor*. These have already been described by Castellani and Willey. I found a similar *Filaria* in the blood of the *Varanus* from Trincomalee.

Miss Robertson (1908) apparently also found no Protozoa in the blood of most Ceylon lizards, for she says: "The common *Calotes* and the beautiful Brahminy lizard ...... and the skink and the horned up-country lizard were all negative, so also the common little house gecko who lives on the wall and eats flies."

Snakes.

I have been able to examine sixteen different species of snakes. As I hope shortly to describe in detail the results of my investigations into the life-histories of the hæmogregarines of Ceylon snakes, I will here give merely a brief record of my observations:—

A.—*Infected.*

1. *Dipsadomorphus forstenii.*—Blood containing hæmogregarines in large numbers. A single individual (Colombo, Aug.).

2. *Dipsadomorphus ceylonensis.*—One individual, slightly infected with hæmogregarines (Peradeniya, Aug.).

3. *Dryophis mycterizans.*—The green whip snake was found to harbour a hæmogregarine (though not invariably) at Colombo (Aug.) and Peradeniya (Sept.). A single individual examined at Trincomalee (Sept.) was negative.

4. *Naia tripudians.*—A single cobra (Peradeniya, Sept.) was infected with hæmogregarines.

5. *Tropidonotus stolatus.*—Out of four individuals examined, two showed no blood parasites (Peradeniya, Aug.; Trincomalee, Sept.). One individual (Colombo, July) had spirochæts in its blood. (See p. 77.) One individual (Peradeniya, Aug.) was infected with trypanosomes (see p. 77) and hæmogregarines.

6. *Zamenis mucosus.*—Rat snakes were always infected with hæmogregarines at Colombo (Aug.) and Peradeniya (Aug.). One individual examined at Trincomalee (Sept.) was not infected.

B.—*Not infected.*

7. *Ancistrodon hypnale.*—Two individuals from Hakgala (Sept.) and one from Kandy (Sept.).

8. *Cerberus rhynchops.*—One individual from Negombo (Sept.) and two from Colombo (Sept.).

9. *Dendrelaphis tristis* (= *Dendrophis pictus*).—Two from Sigiriya (Sept.), one from Trincomalee (Sept.), and one from Peradeniya (Sept.).

10. *Helicops schistosus.*—Two specimens: one small (Colombo, Aug.), the other very large (Colombo, Sept.).

11. *Hydrus platurus.*—A single specimen (Colombo, July).

12. *Lycodon aulicus.*—A single young individual (Colombo, Aug.).

13. *Oligodon sublineatus.*—Four individuals from Peradeniya (Aug. and Sept.).

14. *Python reticulatus.**—A single snake caught in Colombo (July).

15. *Tropidonotus asperrimus* (= *T. piscator*).—One individual from Colombo (Aug.), five from Trincomalee (Sept.), and four from Colombo (Sept.).

16. *Viper russellii.*—A single young specimen (Peradeniya, Sept.).

Comments.—Hæmogregarines have not been previously recorded from *Dipsadomorphus forstenii* or from *D. ceylonensis*. Hæmogregarines are recorded already from *Zemanis mucosus* and *Dryophis mycterizans* in India by Patton (1908), and from *Z. mucosus* in Ceylon by Miss Robertson (1908). The latter also found hæmogregarines in three other Ceylon snakes: *Chrysopelea ornata*, *Naia tripudians*, and "a large python."

A hæmogregarine has been described from *Naia tripudians* by Simond (1901), Laveran (1902), and Patton (1908). Patton (1908) gives a list of eleven Indian species of snake which harbour hæmogregarines.

I did not succeed in finding *Hæmogregarina mirabilis* (Castellani and Willey) in *Tropidonotus asperrimus*.

Birds.

The only bird I examined was a kingfisher shot at Peradeniya. No Protozoa were found in its blood. Castellani and Willey (1905) record *Hæmoproteus* (*Halteridium*) from the blood of crows (*Corvus splendens* and *C. macrorhynchus*), from the babbler (*Crateropus striatus*), and from the owl (*Scops bakkamœna*).

Mammals.

I examined very few mammals. None showed Protozoa in the blood.

Uninfected.

1. *Funambulus palmarum.*—A single individual (Colombo, July).

2. *Lepus nigricollis.*—One young individual (Peradeniya, Sept.).

3. *Pteropus medius.*—A single specimen (Peradeniya, Sept.).

4. *Tragulus meminna.*—Two individuals (Colombo, Aug.). A peculiarity in the blood corpuscles of these animals seems worthy of record. It was found that, although the leucocytes are large, the red corpuscles are extremely small. In fact, I have never encountered such small erythrocytes in any animal before. In *T. meminna* they have a diameter of only about 2·5 μ.

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* This, of course, is not a native Ceylon snake. It is not known how it came to be in Colombo.

B.—Record of Animals examined for Intestinal Protozoa.

An examination of the alimentary canal of various animals was undertaken, in addition to the examination of the blood just recorded. Below are the results. The animals examined were few compared with those in which a blood examination was made.

AMPHIBIA.

1. *Bufo melanostictus.*—I examined the contents of the large intestine in a few individuals both at Colombo and at Peradeniya. In all the animals examined both *Trichomonas* and *Trichomastix* were found. These animals were indistinguishable from *Trichomonas batrachorum* and *Trichomastix batrachorum* which occur in the European frogs and toads. As I have given a detailed description of these forms elsewhere (Dobell, 1909), I will say no more about them here.

A flagellate, which appeared to be identical with the *Octomitus* of the English frog, was also found (*cf.* Dobell, 1909).

In one *B. melanostictus* from Peradeniya a new species of *Nyctotherus* was present. (See p. 75.)

2. *Ixalus leucorhinus.*—A single individual examined in Peradeniya appeared to have absolutely no Protozoa of any sort in its gut.

3. *Rana tigrina.*—Individuals were examined both in Colombo and in Peradeniya. The following Protozoa were found :—

In the large intestine :—An *Entamœba*, indistinguishable from *E. ranarum*, Grassi (*cf.* Dobell, 1909); three flagellates—*Trichomonas*, *Trichomastix*, and *Octomitus*—which appear to be identical with the corresponding organisms in *Rana temporaria* in Europe (*cf.* Dobell, 1909) ; the following Ciliata :—*Opalina*, a small multinucleati species, which was not examined in stained preparations ; large and small *Balantidium* (see p. 74) ; and *Nyctotherus macropharyngeus* (Bezzenberger, 1904). At Peradeniya the oocysts of a coccidian (?) were found. (An examination of the epithelium of the small intestine proved negative.)

In the duodenum :—*Balantidium*, sp. (See p. 74.)

4. *Rhacophorus maculatus.*—This animal was found (Peradeniya) to harbour an *Opalina* and a *Nyctotherus*, both apparently new. (See pp. 76 and 75.) The latter appears to be the same as that found in *Bufo melanostictus* from the same locality.

Remarks.—No intestinal Protozoa seem to have been described from Ceylon frogs and toads hitherto, though Bezzenberger (1904) has described a number of ciliates from various "Asiatic" Anura (localities not given). Further remarks upon the intestinal Protozoa of Amphibia will be found on p. 74.

Lizards.

1. *Hemidactylus leschenaultii.*—One specimen (Habarana) had Trichomonads, and another undetermined flagellate in the large intestine. These were not studied further.

2. *Lygosoma punctatum.*—Several animals were examined at Peradeniya, but no Protozoa were found in the gut.

3. *Lyriocephalus scutatus.*—One individual examined at Peradeniya. Beyond the spores of a coccidian (?) nothing was found. (Epithelium of small intestine negative.)

4. *Mabuia carinata.*—All the individuals examined were found to harbour both *Trichomastix* and *Trichomonas.* (See p. 77.)

Snakes.

I examined only three snakes for intestinal Protozoa:—*Zamenis mucosus* and *Lycodon aulicus* were both negative; *Dryophis mycterizans*, however, contained *Trichomonas* and a *Trichomastix*, which closely resembled the organism which I have already described from *Boa constrictor* (Dobell, 1907). I did not make a careful study of these organisms.

Mammals.

At the instigation of Dr. Willey I made an examination of the contents of the stomach of the two mouse deer (*Tragulus meminna*), which came into my hands at Colombo. In both animals the stomach was literally seething with oligotrichous ciliates, belonging to the family Ophryoscolecidæ, Stein.

These ciliates were discovered by Dr. Willey, but have not as yet been described. At his suggestion I preserved a quantity of the organisms, of which I hope to publish a full description shortly.

Molluscs.

Whilst at Trincomalee, in September, I examined eight species of lamellibranchs, in order to find out whether they harboured spirochæts. These organisms were found in the crystalline style of only two species: *Venus* (*Meretrix*) *casta* and *Soletellina acuminata.*

Dr. Willey had previously noticed spirochæts in these two species, but had not described them. I hope to publish a full account of my observations on these organisms shortly.

Arthropods.

I examined several specimens of the large scorpion, *Palamnœus indus*, in Colombo (July), all with negative results so far as Protozoa were concerned.

Six individuals of the common myriopod, *Polydesmus saussurii*, Humb., collected at Avisawella in July, also showed no Protozoa, though all were infected with a nematode worm, an *Oxyuris* or allied genus.

Another myriopod, *Spirostreptus lunelii*, Humb., from Avisawella (July), also proved negative.

Some white ants, *Calotermes militaris*, from Peradeniya (August), were more interesting. They contained Trichonymphids, a *Nyctoherus*, and a spirochæt, all apparently new. (See p. 80.)

II.—DESCRIPTIONS OF SOME NEW FORMS.

In this part of the paper some of the new, or hitherto undescribed, forms which are recorded in the preceding section are described in greater detail.

The Parasites of Frogs and Toads.

I have already noted the presence of a hæmogregarine in the blood of *Rana tigrina* (p. 67). As I have already remarked, it seems to be identical with *H. berestneffi* of the Indian frog. There is only one other blood parasite which I have to describe.

(1) *Trypanosoma*, sp., of *Rana tigrina*.

This organism resembles the more slender forms of *T. rotatorium* of the European frog. When living the posterior end is bluntly pointed, and the animal usually has a ribbed appearance like that of *T. rotatorium*, but the ribs are only one or two in number (*cf.* fig. 12). There is a well-developed undulating membrane extending about halfway along the organism and ending in a free flagellum of moderate length (*cf.* fig. 12). The trophic nucleus is ovoid, and situated near the anterior end. The kinetic nucleus is a small deeply-staining granule about midway between the trophic nucleus and the extreme posterior end.

Like *T. rotatorium*, this trypanosome is difficult to fix in blood smears. Most of the stained specimens which I obtained were badly distorted. Fig. 12 is drawn from one of the most favourable fixed and stained animals which I encountered, but it makes the animal appear a good deal stouter than it appears when alive.

The average length of the trypanosome, so far as I have been able to determine from the few well-preserved specimens which I obtained in my preparations, is between 30 μ and 40 μ, including the free flagellum.

Balantidium.

As already recorded, I found species of *Balantidium* inhabiting the duodenum and the large intestine of *Rana tigrina*.

(2) *Balantidium ovale*, n. sp.

This name I propose for the common species of *Balantidium* which occurs in the large intestine of *R. tigrina*. The animal is very like several other species already described. It differs from *B. helenæ*, Bezzenberger, only in size. Bezzenberger describes this species as occurring in *R. tigrina*, *R. cyanophlyctis*, *R. limnocharis*, and *R. hexadactyla*, but he does not state from what parts of Asia the frogs came. He gives 110 μ × 60 μ as the average dimensions. The average size of my forms, however, is about 80 μ × 50 μ. Apart from this, Bezzenberger's description of *B. helenæ* applies equally well to *B. ovale*. The peristome has the same form, the meganucleus is kidney-shaped, lying posteriorly, with the micronucleus in the hollow. I have omitted to figure the organism, as Bezzenberger's figure of *B. helenæ* is almost identical.

I found numerous animals undergoing division, and also found encysting and encysted forms. These present no essential differences from what has already been described in other members of the genus. The cysts are ovoid, and measure ca. 54 μ × 44 μ.

In addition to the large forms just described, I found numerous smaller forms—also dividing actively—which were identical in every way except in size. They were only about two-thirds the size of the larger animals. Whether these represent another species or not, I am unable to decide.

(3) *Balantidium hyalinum*, n. sp.

I propose this name for the organism which occurs in the duodenum of *R. tigrina*. It does not differ markedly from other duodenal forms, namely, *B. duodeni*, Stein (in *Rana esculenta* and *R. temporaria*), and *B. rotundum*, Bezzenberger (in *R. esculenta*, var. *chinensis*). It is often present in large numbers in the small intestine, and when alive its protoplasm is more hyaline than that of any other *Balantidium* which I have seen.

The organism (fig. 19) is oval, with a straight mouth extending almost to the middle of the body. The meganucleus is posteriorly placed, and is ovoid. The micronucleus can nearly always be seen at one end of the meganucleus, not in the middle (*cf.* fig. 19). There is one contractile vacuole. In the anterior region the curious striated or granular triangular area, which is characteristic of *B. duodeni* and *B. rotundum*, is usually clearly seen (see fig. 19). As in these forms also, the cilia are long and well developed over the whole body. The average dimensions are ca. 74 μ × 56 μ.

(A curiously long and slender form has been described by Bezzenberger—under the name *B. gracilis*—from the small intestine of *Rana hexadactyla* and *R. cyanophlyctis*.)

NYCTOTHERUS.

In addition to *Nyctotherus macropharyngeus*, Bezzenberger,* which I found in *R. tigrina* in Colombo, I found a species of *Nyctotherus* in *Bufo melanostictus* and *Rhacophorus maculatus* at Peradeniya. It appears to be the same species in both hosts, and I propose to name it—

* This is a very large species. Its most striking feature is its very long and spirally wound pharynx.

(4) *Nyctotherus papillatus*, n. sp

The animal has the usual reniform appearance characteristic of the genus. Those taken from the large intestine of *B. melanostictus* measured ca. 120 μ in length, whilst those from *R. maculatus* were distinctly larger, the largest attaining a length of 170 μ. In other respects they were identical.

The pharynx extends to the median line, is sharply curved into an almost perfect semi-circle, and has a well-marked spiral twist. The anus opens just dorsally to a well-marked papilla at the extreme posterior end of the animal. There is one contractile vacuole, situated close to the anus. The meganucleus is in the usual position anteriorly, but appears to be reniform or horseshoe-shaped, with the ends directed ventrally, so that it appears to be ovoid when seen from the side. A micronucleus was not always seen, but was sometimes visible lying on the meganucleus.

A curious little diverticulum of the pharynx just at its point of junction with the mouth was nearly always observable. It passes dorso-posteriorly for a very short distance, and then appears to end blindly. I have never seen this curious little structure in other species of the genus.

OPALINA.

Rana tigrina, as I have already noted, was found to harbour a multinucleate species of *Opalina*, which I observed in the living state only. A pretty species of *Opalina* was found in *Rhacophorus maculatus* at Peradeniya, and I was able to study it more carefully. As it seems to be new I propose the name—

(5) *Opalina virgula*, n. sp.,

for the organism. Its characteristics are as follows. The general shape of the body is that of a large flattened comma; that is to say, there is a large bulge on one side anteriorly (see fig. 17). It thus resembles *O. obtrigona* (parasitic in the European tree frog *Hyla arborea*) more closely than any other of the dozen or so species of *Opalina* hitherto described.* Some of the individuals are long and slender, and others are stouter and more rounded, but all have this general appearance. The body is flattened, *i.e.*, elliptical in transverse section, and the cilia are distributed over the body in lines, as in other species. Large individuals may measure 170 μ, or rather over, in length, and 50 μ in breadth at the broadest part of the anterior end.

The animal is multinucleate. All the nuclei in my preparations (picro-acetic acid, Delafield's hæmatoxylin) appear as rather loose masses of chromatin granules (see fig. 17). Other slightly stained bodies are also present in the endoplasm. They appear to be the bodies which Metcalf calls "endosarc spherules," and which occur in other *Opalinæ*.

* *Cf.* Metcalf's (1909) recent monograph on the genus.

In company with these larger forms were a number of smaller forms. These I take to be young forms. They are the shape of a flattened spindle, and contain few nuclei (see fig. 18). Possibly they are organisms which are on their way to encystment. The small form figured (fig. 18) measured 38 $\mu \times 13\ \mu$.

Bezzenberger (1904) has described *Opalinæ* from *Bufo melanostictus*, *Rana cyanophlyctis*, *R. limnocharis*, *R. hexadactyla*, and *R. esculenta*, var. *chinensis*, but he does not state from what part of Asia these animals came.

Intestinal Parasites of Lizards.

Parasitic flagellates were found in the gut in only two lizards: *Hemidactylus leschenaultii* and *Mabuia carinata*. Both these hosts contained both *Trichomonas* and *Trichomastix*, but a careful study was made of those in *Mabuia* only.

{ *Trichomonas mabuiæ*, n. sp.
{ *Trichomastix mabuiæ*, n. sp.

I have elsewhere described (Dobell, 1909) in detail the structure of *Trichomonas* and *Trichomastix batrachorum*. The two organisms from *Mabuia* have a structure which is exactly similar. My chief reason for noting these organisms here is that they furnish a striking confirmation of what I have already described in the structure of the frog and toad parasites.

Trichomonas mabuiæ (fig. 11) attains a length of 30 μ, and it is quite easy to observe *in the living animal*, under an oil immersion, all the details of structure which I have already described in the much smaller *T. batrachorum*. Structures which, in the latter, were frequently only made out in stained preparations, and with considerable difficulty, can be seen in *T. mabuiæ* with the greatest clearness. The relations of the nucleus, axostyle, blepharoplast, and undulating membrane are exactly as I have already described them. To describe the forms from *Mabuia* would be merely to repeat what I have already written. I will therefore content myself with figuring *Trichomonas mabuiæ*, and would refer any one interested in the structure of these organisms to my earlier paper.

The Parasites of Tropidonotus stolatus.

As recorded on p. 70, I found three parasites in the blood of this snake: a hæmogregarine, a spirochæt, and a trypanosome. The last two are new; the first is probably the same as the "*Danilewskya*" described in *T. stolatus* from Tonkin by Billet (1895). [*Cf.* also Dobell (1908).]

(1) *Trypanosoma tropidonoti*, n. sp.

I propose to give this name to the new trypanosome which I found in the blood of a *T. stolatus* at Peradeniya (see figs. 13, 14).

When observed in the fresh blood of the snake the organism exhibited no characteristics which would distinguish it readily from many other trypanosomes. It was actively motile, with a short free flagellum terminating the undulating membrane, which extended along about half the length of the body. The posterior (aflagellar) half of the body was drawn out to a sharp point. Though the trophic nucleus was easily visible in the living animal, the kinetic nucleus was observed only after staining. The cytoplasm was finely granular in appearance and uniform throughout.

In smears stained by Giemsa's method, the following structure was observable (see figs. 13, 14):—

The body is sharply pointed at both ends, with the trophic nucleus lying near the middle as a homogeneous pink mass of granules. The flagellum and undulating membrane appeared the same as in the fresh preparations, but the kinetic nucleus, with the origin of the membrane, &c., could now be made out accurately. The kinetic nucleus itself is a small granule staining a deep purple with Giemsa's stain. It is remarkable on account of its position. Sometimes it was situated well behind the trophic nucleus (fig. 13), but at other times it was placed actually in contact with it (fig. 14). Intermediate positions were also seen. The latter arrangement, *i.e.*, in contact with the trophic nucleus, gives the animal an appearance suggesting an organism which is halfway between a *Crithidia* and a *Trypanosoma*. The average length of the organism (including the free flagellum) is 30 μ–40 μ.

So far as I am aware, only two trypanosomes have been recorded from snakes hitherto: *T. erythrolampri* (Wenyon, 1908) from *Erythrolamprus æsculapii* (tropical America), and *T. naiæ* (Wenyon, 1908*a*) from *Naia nigricollis* (Africa). Only one of these was satisfactorily investigated as regards its nuclear apparatus (*T. erythrolampri*), and it is a curious fact that it shows the same peculiarity which I have pointed out above in the case of *T. tropidonoti*. The two organisms are, in fact, very closely similar in other respects also.

Another trypanosome in which the kinetic and trophic nuclei are in close proximity has recently been described—under the name *T. pertenue*—by Miss Robertson (1908) from the blood of the Ceylon geckoes, *Hemidactylus triedrus* and *H. leschenaultii*.

(2) *Spirochæta tropidonoti*, n. sp.

This is the first record of a spirochæt from the blood of a snake. It is therefore much to be regretted that my observations on it are exceedingly scanty.

Only a single *T. stolatus* was found harbouring the organism. In the fresh blood preparations the spirochæts were rare, and in the stained smears made from the same blood they were still more difficult to find. Through a most unfortunate accident most of my stained preparations were lost before they had been carefully examined.

No ticks were found on the snake, but one is tempted to suggest that these animals, which are common on many snakes, are the carriers of the spirochæt.

The living spirochæts (fig. 15) appeared as slender, flexible, corkscrew-like organisms, actively motile. and closely resembling *S. duttoni* in general form. In length they measure ca. 15 μ, and their breadth is probably about 0·5 μ, though I have not been able to obtain sufficiently accurate measurements of the latter.

In the films stained by Giemsa's method the organisms were coloured a uniform pink.

In a single instance (fig. 16) I observed an organism which appeared to be on the point of dividing into two. But whether division had been longitudinal or transverse it was impossible to decide. The thickness of the organism certainly suggests the latter mode of division.

The Hæmogregarine from Crocodilus porosus.

Hæmogregarines have already been described from crocodiles in various parts of the world. Simond (1901*a*) appears to have been the first to record hæmogregarines from Crocodilia. He described (1901, 1901*a*) a form, under the name *H. hankini*, from the Indian gavial; and he further noted (1901, p. 320) that the same organism occurred in *Crocodilus porosus* (?), and stated that Marchoux had found a similar parasite in a Senegal crocodile.

Börner (1901) almost simultaneously described a hæmogregarine from *Crocodilus frontatus* and *Alligator mississippiensis*, and gave it the name *H. crocodilinorum*. If these prove to be the same species, then the priority of name rests with *H. hankini;* for, as Simond points out, his account was published a month before that of Börner. It is probable, therefore, that the Ceylon form from *C. porosus* is *Hæmogregarina hankini*, Simond.

Minchin, Gray, and Tulloch (1906) figure a hæmogregarine from a Central African crocodile, and this organism is repeatedly mentioned in subsequent reports of various sleeping sickness commissions.

The form which I found in the Ceylon crocodile bears a close resemblance to many of the figures of Simond and Börner.

All the individuals which I examined were in red blood corpuscles from the circulating blood. They all presented the appearance shown in figs. 9, 10; that is to say, they were all large, doubled-up individuals. Sometimes the two halves were approximately equal in thickness (fig. 9), but sometimes one was considerably thicker than the other (fig. 10). In preparations stained by Giemsa's method the nucleus always appeared as a compact mass of deep purple granules (figs. 9, 10). In length the animals (doubled up) measured from 12 μ to 15 μ.

In the absence of more material, I can do little more here than record and figure the organism.

The Parasites of White Ants.

(1) *Gymnonympha zeylanica*, n. g., n. sp.

As already recorded (p. 74), I found the termites* (*Calotermes militaris*) which I examined at Peradeniya infected with a protozoon belonging to the family Trichonymphidæ.

The Trichonymphids are characterized by possessing a large number of flagella, which originally gave rise to their inclusion among the Ciliata. [See Butschli (1887), S. Kent (1882), &c.] I have little doubt, however, that they are really referable to the Mastigophora (*cf.* Doflein, 1909). It is curious to note that Leidy (1877), who first gave us an accurate description of these organisms, remarks—speaking of *Trichonympha*—that they are "of obscure affinity, but probably related with the Turbellaria on the one hand, and by evolution with the Ciliate Infusoria on the other."

The organisms which I found in Ceylon do not appear to belong to any of the genera hitherto described. [See Leidy (1881), Grassi (1888), Grassi and Sandias (1893), Frenzel (1891).] *Leidyonella* (Frenzel, 1891) is the form which appears to approximate most nearly to my organisms.

As far as I am aware, no Trichonymphids have been described from Asiatic white ants before, but it seems highly probable that these parasites occur in white ants throughout the world. They were apparently discovered by Lespes in Europe in 1856, and were subsequently described in North America (Leidy), in the Argentine (Frenzel), and in Europe (Grassi and others). The closely allied form *Lophomonas* is a frequent parasite of the common cockroach, *Stylopyga orientalis*.

Gymnonympha zeylanica, as I propose to name the new organism, is distinguished by possessing comparatively few flagella, which are confined entirely to the anterior end of the body, as in *Jœnia* and *Lophomonas*, but there is no axostyle present.

The general form of the animal (see fig. 1) is roughly ovoid or pyriform, but the body is so plastic that its shape is constantly undergoing change during life. At the extreme anterior end the body is drawn out into a small conical process surrounded by a curious vesicular cap (fig. 1). Where the cap unites, by its edges, with the conical process, the flagella arise, apparently in a single ring round the base of the cone. The length of the largest forms is about 150 μ. The flagella measure only about one-half of the length of the body. Running backwards from the point of origin of the flagella, a series of striations can be seen extending for about one-third of the length of the organism. These striations appear to be situated in the investing cuticle.

* All the individuals examined were workers.

The nucleus is round, and measures about 15 μ in diameter. It is composed of a mass of small chromatin granules surrounded by a clear achromatic membrane. It usually lies at the anterior end of the animal.

Inside the body, especially in the posterior region, a number of particles of wood can usually be seen. How they are ingested I am unable to say, as I have never observed an animal in the act of taking them up, nor is a mouth present, as far as I have been able to make out.

In addition to these larger forms just described, I always found smaller animals possessing a somewhat different structure. The anterior end and arrangement of the flagella was different, and the nucleus was situated posteriorly (fig. 2). I think these small forms probably represent young stages in the life-history of *Gymnonympha*, but in the absence of any very definite intermediate forms, I must leave this an open question for the present. These small forms were usually about 30–40 μ in length.

No animals in division, or at different stages in the life-cycle, have I been able to find.

(2) *Nyctotherus termitis*, n. sp.

I propose this name for the new species of ciliate which I found in the termite. Hitherto no *Nyctotherus* has, I believe, been recorded from white ants.

N. termitis differs but slightly from several other members of the genus. It resembles *N. ovalis*, Leidy, of the common cockroach (*Stylopyga orientalis*) closely in general structure. The body is roughly ovoid, with the gullet situated near the middle, and running in obliquely with a very slight curvature (see fig. 21). It does not extend more than about halfway across the animal. There is a well-marked, though narrow, anus, near to which—on the ventral side—the single contractile vacuole is situated (see fig. 21). The meganucleus is ovoid or slightly horseshoe-shaped, and a micronucleus can sometimes be seen lying in close contact with it. At the level of the meganucleus the body shows a more or less strongly marked constriction. Another similar constriction can be seen about halfway between this and the extreme anterior end. (*Cf.* fig. 21.)

The animal attains a length of 60–70 μ, and a maximum breadth of rather more than 40 μ.

It is rather a striking fact that the white ant should harbour a *Nyctotherus* so closely resembling that of the cockroach, when it is remembered that the Trichonymphids are also confined to these two hosts.

(3) *Spirochæta termitis*, n. sp.

Some of the termites which I examined proved to be heavily infected with spirochæts. As these have not been previously described—so far as I am aware—I propose the name *S. termitis* for them.

It is of interest to note that Leidy (1877) found "a Spirillum" present in the gut of *Termes flavipes* (North America); and Grassi and Sandias (1893) also record "Spirilla" in the European termites which they investigated (*Calotermes flavicollis* and *Termes lucifugus*). It seems to me highly probable that reinvestigation of these organisms would show them to be really spirochæts.*

When alive, *S. termitis* is a long, slender, and very active organism. It moves rapidly backwards and forwards with the wriggling, flexible motion characteristic of the spirochæts. A well-marked bending and rolling up of the body may frequently be seen.

The organisms which I observed (fig. 20) varied considerably in size, both as regards length and breadth. The longest individuals measured rather over 60 μ, but the breadth was never more than 1 μ, and often less.

The ends are pointed, and do not appear to bear free flagellar processes, such as are said to occur in some spirochæts (*e.g.*, *S. buccalis*). Neither in the living organism nor in stained preparations have I seen an undulating membrane.

In films stained by Giemsa's method the organisms stained a uniform pink, or occasionally showed an indistinct granular structure. Owing to their slenderness it is exceedingly difficult to make out their internal structure.

I found no forms which could be regarded with certainty as showing stages in division, though some of the longest organisms—in stained preparations—occasionally exhibited a break towards the middle of the body (fig. 20, longest individual), which suggested that transverse division takes place.

Concluding Remarks.

I wish, in conclusion, to summarize some of the more interesting points which the observations recorded in the foregoing pages have brought to light.

In the first place, I would emphasize the fact that my investigations are not, and do not pretend to be, in any way exhaustive. I have merely examined such animals as chance allotted to me. Also in no case did I examine more than a small number of individuals of any one species. Many animals, moreover, were examined with entirely negative results, and I am fully sensible of the fact that no definite deductions can be drawn from these few negative instances. The record of these cases has been given solely for the use of subsequent workers along similar lines. Nevertheless, apart

* Since writing the above, I have been able to consult the full account of these organisms by Leidy (1881). His description and figures leave no doubt in my mind that his organisms were really spirochæts. Curiously enough, he has named the organisms *Vibrio termitis*. If mine are the same as the North American forms, the correct name is therefore *Spirochæta termitis*, Leidy emend. Dobell (non *Sp. termitis*, Dobell).

from these inconclusive negative results, I have obtained a few positive records, which appear to me to justify a few general remarks before I conclude.

A point of some interest is in connection with the distribution of the protozoan parasites is frogs. I have found, as recorded in previous pages, that the Ceylon frogs harbour a set of Protozoa exactly parallel to the set which one finds in European frogs. In both one finds two kinds of blood Protozoa: Hæmogregarines and Trypanosomes. In both one finds three genera of flagellates in the large intestine: *Trichomonas*, *Trichomastix*, and *Octomitus* (*Hexamitus*). In both one finds an *Entamœba* in the large intestine. In both one finds ciliates—belonging to the three genera: *Opalina*, *Balantidium*, and *Nyctotherus*—in the large intestine. In both, finally, one finds a ciliate of the genus *Balantidium* inhabiting the duodenum. The three flagellates and the amœba correspond in general appearance so closely in the Ceylonese and European frogs that I cannot distinguish them from one another.

Again, the Ceylon crocodile has been found to harbour a hæmogregarine, which resembles not only that described from the Indian gavial, but also those found in African crocodiles and the Mississippi alligator.

Then in the snakes. Apart from the new spirochæt which was found, one finds hæmogregarines which resemble not only those found in snakes from other parts of Asia (India, Tonkin, China, Java, &c.), but also those in snakes from Europe, from Africa, from North and South America, and from Australia. The only snake trypanosome which I found is closely similar to another previously described from a tropical American snake. Further, one Ceylon snake was found to possess a *Trichomastix* very like that which I have already described from a South American *Boa constrictor*. A similar organism occurs in all probability in European snakes.

Then, in the case of the white ants, similar interesting finds have been recorded. Ceylon termites harbour a flagellate belonging to the remarkable family Trichonymphidæ. These parasites have previously been found in termites in Europe, North America, and South America. The only other host of trichonymphids is the cockroach. It is therefore of interest to find that the Ceylon termite harbours a ciliate of the genus *Nyctotherus*, which very closely resembles that of the common cockroach, *Stylopyga orientalis*. This is a fact not without interest for the systematic entomologist. Lastly, the Ceylon termite possesses a spirochæt, and there are indications that the North American and European termites harbour a similar parasite.

Some further parallels could be added to this list, but it is perhaps unnecessary to develop this theme any further. Yet it seems to me that these facts are of something more than purely protozoological interest.

It will not perhaps be superfluous to point out once more that I have, in company with other workers on Indian and Ceylon forms, found that lizards do not appear to be infected with blood Protozoa to anything like the same extent that European and African lizards are.

One other point, in conclusion, appears to me worthy of comment. Wherever I have found trichomonads, I have always found both *Trichomonas* and *Trichomastix* associated together. This supports, I think, to some extent the view of Doflein, who believes that these two "genera" are in reality merely forms of one and the same organism. For my own part, however, I prefer to consider them as distinct genera for the present, mainly on the ground that no real intermediate forms have ever been discovered. This is, however, a matter of but small importance.

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DESCRIPTION OF PLATE.

(The figures are not drawn to scale. The actual dimensions of the various organisms depicted are given in the text. Figs. 1, 2, and 20 were drawn under Zeiss 3 mm. apochromatic oil immersion × comp.-oc. 12. Figs. 3–10 and 12–15 were drawn under Leitz 1/12 in. oil immersion. Figs. 11, 16–19, and 21 were drawn under Zeiss 2 mm. apochromatic oil immersion, comp.-oc. 6.)

Fig. 1.—*Gymnonympha zeylanica*, n. g., n. sp., a trichonymphid from the intestine of *Calotermes militaris*. (Picro-acetic acid, Delafield's hæmatoxylin and eosin.)

Fig. 2.—Small trichonymphid, from same preparation as preceding. (Probably a young form ?)

Figs. 3–8.—*Hæmogregarina*, sp., from *Rana tigrina*. (Osmic vapour, Giemsa.)

Fig 3.—Large intracorpuscular form.

Figs. 4, 5, 6.—Various free forms from the blood plasma.

Fig. 7.—Encapsuled form in a red blood corpuscle. (Living animal.)

Fig. 8.—Empty sheath of parasite lying in red corpuscle. (Dry film ; absolute alcohol, Giemsa.)

Figs. 9, 10.—*Hæmogregarina*, sp. (? *H. hankini*, Simond), from blood of *Crocodilus porosus*. (Dry films ; absolute alcohol, Giemsa.)

Fig. 9.—Doubled-up organism, with limbs approximately equal in thickness.

Fig. 10.—Form with slender doubled-up " tail."

Fig. 11.—*Trichomonas mabuiæ*, n. sp., from the large intestine of *Mabuia carinata*. Large individual. (Sublimate alcohol, Delafield's hæmatoxylin and eosin.)

Fig. 12.—*Trypanosoma*, sp., in blood of *Rana tigrina*. (Osmic vapour, Giemsa.) A red corpuscle is shown in outline.

Figs. 13 and 14.—*Trypanosoma tropidonoti*, n. sp., from the blood of *Tropidonotus stolatus*. (Osmic vapour, Giemsa.) The body is stained blue, the trophic nucleus pink, edge of membrane with flagellum red, the kinetic nucleus deep purple.

Fig. 13.—A form in which the kinetic nucleus is situated some distance posterior to the trophic nucleus. (Red corpuscle in outline.)

Fig. 14.—Form in which the kinetic nucleus is in contact with the trophic nucleus.